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*J Neurophysiol* 100:1983-1994, 2008. First published 13 August 2008; doi:10.1152/jn.90635.2008

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# Differential Activity-Dependent, Homeostatic Plasticity of Two Neocortical Inhibitory Circuits

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Submitted 3 June 2008; accepted in final form 7 August 2008

**Bartley AF, Huang ZJ, Huber KM, Gibson JR.** Differential activity-dependent, homeostatic plasticity of two neocortical inhibitory circuits. *J Neurophysiol* 100: 1983–1994, 2008. First published August 13, 2008; doi:10.1152/jn.90635.2008. Chronic changes in neuronal activity homeostatically regulate excitatory circuitry. However, little is known about how activity regulates inhibitory circuits or specific inhibitory neuron types. Here, we examined the activity-dependent regulation of two neocortical inhibitory circuits—parvalbumin-positive (Parv+) and somatostatin-positive (Som+)—using paired recordings of synaptically coupled neurons. Action potentials were blocked for 5 days in slice culture, and unitary synaptic connections among inhibitory/excitatory neuron pairs were examined. Chronic activity blockade caused similar and distinct changes between the two inhibitory circuits. First, increases in intrinsic membrane excitability and excitatory synaptic drive in both inhibitory subtypes were consistent with the homeostatic regulation of firing rate of these neurons. On the other hand, inhibitory synapses originating from these two subtypes were differentially regulated by activity blockade. Parv+ unitary inhibitory postsynaptic current (uIPSC) strength was decreased while Som+ uIPSC strength was unchanged. Using short-duration stimulus trains, short-term plasticity for both unitary excitatory postsynaptic current (uEPSCs) and uIPSCs was unchanged in Parv+ circuitry while distinctively altered in Som+ circuitry—uEPSCs became less facilitating and uIPSCs became more depressing. In the context of recurrent inhibition, these changes would result in a frequency-dependent shift in the relative influence of each circuit. The functional changes at both types of inhibitory connections appear to be mediated by increases in presynaptic release probability and decreases in synapse number. Interestingly, these opposing changes result in decreased Parv+-mediated uIPSCs but balance out to maintain normal Som+-mediated uIPSCs. In summary, these results reveal that inhibitory circuitry is not uniformly regulated by activity levels and may provide insight into the mechanisms of both normal and pathological neocortical plasticity.

## INTRODUCTION

Neocortical activity levels are chronically altered during sensory map plasticity (Horton and Hubel 1981), neuronal circuit maturation (Turrigiano and Nelson 2004), and pathological conditions, such as epilepsy or stroke. To understand how activity modifies neural circuit properties during these conditions, we must know the cellular alterations occurring in different cell types. More specifically, we must know the role played by inhibitory neurons since they greatly influence network properties by controlling action potential generation and synaptic integration.

Across various species, most adaptations in neuronal properties in response to chronic activity level changes (minutes to

days) appear to be homeostatic (Davis and Goodman 1998; Marder and Prinz 2002; Turrigiano and Nelson 2000). Regulation of cortical excitatory neurons, via synaptic and intrinsic membrane alterations, is usually consistent with the homeostatic maintenance of activity levels at a particular set point (Desai et al. 1999b, 2002; Hartman et al. 2006; Hendry and Jones 1988; Kilman et al. 2002; Lissin et al. 1998; Marty et al. 2000; Micheva and Beaulieu 1995; Murthy et al. 2001; Turrigiano and Nelson 2000; Turrigiano et al. 1998). For example, excitatory drive onto individual excitatory neurons is increased after chronic action potential blockade. This is homeostatic because action potential generation is facilitated to regain the set point for average activity.

While the activity-dependent regulation of neocortical inhibitory synapses has been commonly studied (Chattopadhyaya et al. 2004; Hartman et al. 2006; Kilman et al. 2002; Maffei et al. 2004, 2006; Marty et al. 1997; Patz et al. 2003; Welker et al. 1989), little is known about how homeostasis is maintained in inhibitory neurons themselves. Unlike excitatory neurons, excitatory drive onto inhibitory neurons has been reported to be unchanged after activity blockade (Turrigiano et al. 1998). Similar to excitatory neurons, inhibitory neurons increase their intrinsic excitability in response to activity blockade (Desai et al. 1999a; Gibson et al. 2006).

However, neocortical inhibitory neurons are divided into subtypes which likely subserve different functions (Gibson et al. 1999; Reyes et al. 1998; Somogyi et al. 1998), and previous electrophysiological studies examining the homeostatic regulation of inhibitory neurons and their synapses have usually not made this distinction (but see Maffei et al. 2004). Due to their different roles in circuit function, different inhibitory subtypes and their synapses may be regulated differently by chronic changes in activity. Therefore important questions remain. To what degree do different inhibitory neuron subtypes display homeostatic regulation of firing rate? To what degree are different synapse types differentially regulated by activity? Are the cellular mechanisms used to maintain homeostasis in different cell and synapse types similar?

Here we investigated activity-dependent regulation of synapses involving two inhibitory subtypes—parvalbumin-positive (Parv+) and somatostatin-positive (Som+) neurons (Gonchar and Burkhalter 1997). These two subtypes have distinctly different electrophysiological and anatomical properties that suggest different roles in neocortical function. To determine how activity levels regulate the local functional

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connectivity to and from Parv+ and Som+ neurons, we performed dual recordings of synaptically coupled neuron pairs in neocortical slice cultures during chronic action potential blockade. The slice culture preparation effectively preserves the three-dimensional structure, the different cell types, and the synaptic development that exists in cortical circuits in vivo (Chattopadhyaya et al. 2004; De Simoni et al. 2003; Gähwiler et al. 1997; Gorba et al. 1999; Stoppini et al. 1991) while also allowing control over activity levels. This experimental design provides insight into the regulation of local “recurrent” inhibition—the disynaptic pathway that includes the excitatory connection to the inhibitory neuron and the inhibitory connection back to the excitatory neuron.

## METHODS

### *Animals and cell identification*

Inhibitory neurons were identified by GFP fluorescence. In one line, GFP was only expressed in a subset of neocortical parvalbumin-positive (Parv+) inhibitory neurons (G42) (Chattopadhyaya et al. 2004). In another line, GFP was expressed only in a subset of somatostatin-positive (Som+) neurons (GIN mice, Jackson Laboratories) (Gibson et al. 2006; Oliva et al. 2000). The use of these mice for studying these biochemically defined neocortical inhibitory subtypes has been previously established (Chattopadhyaya et al. 2004; Di Cristo et al. 2004; Gibson et al. 2006; Oliva et al. 2000). We have previously confirmed the somatostatin expression of GFP neurons in GIN mice in our neocortical slice culture preparation (Gibson et al. 2006). In this study, no GFP-positive neurons ever elicited a unitary excitatory postsynaptic current (uEPSC). In all dual recordings, the two GFP identified inhibitory subtypes displayed properties of action potential generation and afferent uEPSCs consistent with previous studies of the two subtypes (Gibson et al. 1999, 2006; Reyes et al. 1998).

We acknowledge that these two categories may include different morphological subtypes (Dumitriu et al. 2007; Gupta et al. 2000). But when considering the consistent firing and synaptic properties that differentiate these two biochemical subtypes and the abundant anatomical and electrophysiological studies using these categories (Chattopadhyaya et al. 2004; Gibson et al. 1999, 2006; Gonchar and Burkhalter 1997; Oliva et al. 2002; Reyes et al. 1998), this is a useful categorization.

### *Slice culture and pharmacological treatments*

Preparation of interface slice cultures was based on a previous study (Stoppini et al. 1991). Mice (postnatal day 5–7; P5–7) were anesthetized with halothane in a manner consistent with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association. The brain was removed and then dissected in a HEPES-based buffer containing kynurenic acid (1 mM) to obtain a square sheet of somatosensory neocortex, 2–3 mm on each side which was subsequently sliced into 400- $\mu$ m slices with a McIlwain chopper. Slices were transferred to 4°C culture media and then plated onto semiporous membranes (Millicell, Millipore) in warmed culture media. Slices were placed and maintained in an incubator at 5% CO<sub>2</sub>/35°C. Culture media was exchanged the next day and every 2 days thereafter. The first two exchanges involved adding a mitotic inhibitor to the culture medium (FUDR, 35  $\mu$ M; uridine, 80  $\mu$ M). Culture media (based on Musleh et al. 1997) was 20% adult horse serum (Hyclone, defined, SH 30074.02) and 80% MEM (GIBCO, 51200-020) and contained the following (in mM): 1 glutamine (Glutamax, Invitrogen), 0.7 ascorbic acid, 1 CaCl<sub>2</sub>, 2 MgSO<sub>4</sub>, 12.9 dextrose, 5.3 NaCO<sub>3</sub>, and 30 HEPES and 1  $\mu$ g/ml bovine insulin (Sigma), pH 7.3, 315 mosM. Slice culture age was termed “equivalent day” (ED) which

is the sum of “days in vitro” (DIV) and the postnatal age (P) of the dissected mouse.

Chronic activity-blockade was mediated by 5-day tetrodotoxin (TTX, 2  $\mu$ M) treatment beginning at ED15–20. Slices were refreshed with TTX once per day. After 5 days of blockade, neurons appeared as healthy as controls. Images of live slice cultures under DIC microscopy did not appear any different. Electrophysiological properties were normal since neurons had stable, subthreshold resting potentials, fired action potentials, displayed spontaneous synaptic events, and displayed evoked synaptic transmission. This is consistent with another study using 6-day TTX treatment of cortical slice cultures which reported no change in pyramidal cell density using anatomical markers (Chattopadhyaya et al. 2004).

As in other studies and other culture preparations, we have previously shown that these neocortical slice cultures display spontaneous activity and that chronic TTX application increases this spontaneous activity (Echevarria and Albus 2000; Gibson et al. 2006; Turrigiano 1999). Frequency of spontaneous population events was similarly high in the present study when we made no effort to suppress it (~1 event/30 s). This spontaneous activity might affect synaptic measurements so we added compounds to suppress activity (see *Evoked unitary responses*).

### *Electrophysiology*

Unitary excitatory postsynaptic currents (uEPSCs) and unitary inhibitory postsynaptic currents (uIPSCs) were measured using simultaneous (dual) whole cell recordings in cultured slices at 21°C in a submersion recording chamber. Slice age was ED20–25 unless stated otherwise, and all recordings were in layer 2/3. Neurons were within 20  $\mu$ m of each other and typically 8–20  $\mu$ m below the surface. Recordings were performed with IR-DIC visualization (Stuart et al. 1993) using a Nikon E600FN microscope and a CCD camera (Hamamatsu). Resting membrane potential and series resistance were continuously measured to monitor recording stability. Cell capacitance was always measured at recording onset (filtered at 30 kHz, sampled at 50 kHz). Capacitance and input resistance were measured in voltage clamp with a 400 ms, –10-mV step from a –60-mV holding potential. The capacitance was calculated by first obtaining the decay time constant of a current transient induced by the –10-mV step (the faster time constant of a double-exponential decay fitted to the 1st 60 ms) and then dividing this by the series resistance. Data were not corrected for junction potential.

### *Electrophysiology solutions*

Artificial cerebrospinal fluid (ACSF) contained (in mM) 126 NaCl, 3 KCl, 1.25 NaH<sub>2</sub>PO<sub>4</sub>, 2 MgSO<sub>4</sub>, 26 NaHCO<sub>3</sub>, 25 dextrose, and 2 CaCl<sub>2</sub>. Drugs were added to the ACSF to suppress activity (see *Evoked unitary responses*). ACSF is saturated with 95% O<sub>2</sub>-5% CO<sub>2</sub>. The following were the pipette solutions (in mM). K-Gluc: 130 K-gluconate, 6 KCl, 3 NaCl, 10 HEPES, 0.2 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, 14 phosphocreatine-Tris, and 10 sucrose; Cs-Meth: 140 Cs-methanesulfonate, 10 HEPES, 2.5 bis-(*o*-aminophenoxy)-*N,N,N',N'*-tetraacetic acid, 4 ATP-Mg, 0.3 GTP-Tris, 14 phosphocreatine-Tris, 10 sucrose, 2 QX-314-Cl, and 2 TEA-Cl; K-Gluc (high Cl): 80 K-gluconate, 32 KCl, 6 NaCl, 10 HEPES, 0.1 EGTA, 4 ATP-Mg, 0.3 GTP-Tris, 14 phosphocreatine-Tris, and 15 sucrose. Junction potentials were 9, 10, and 7 mV, respectively. Data were not corrected for junction potential. All pipette solutions were adjusted to pH 7.25, 290 mosM.

### *Evoked unitary responses*

For all uPSCs, the presynaptic cell was recorded with K-Gluc pipette solution. The standard presynaptic action potential (AP) train protocol was a five-pulse, 20-Hz train applied every 8 s. Presynaptic action potentials were evoked in voltage clamp by a +20- to +40-mV

step for 8 ms and could be evoked probably because of the inability to voltage clamp at the AP initiation sites.

For uEPSCs, Cs-Meth pipette solution was used in the postsynaptic inhibitory neuron to reduce effects of intrinsic membrane alterations induced by TTX treatment. As a result of prolonged TTX treatment the spontaneous activity of the slices was high, therefore the following were added to the ACSF to decrease spontaneous activity: NBQX (0.05  $\mu\text{M}$ ), zolpidem (0.1  $\mu\text{M}$ ), and 2-amino-5-phosphonopentanoic acid (AP5, 100  $\mu\text{M}$ ). The 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzof[quinoxaline-2,3-dione (NBQX) reduced excitatory responses by ~15–20% (Kumar et al. 2002; unpublished observations). The effects of TTX on synaptic parameters were not dependent on these additions to the ACSF, because similar results were obtained if recordings were performed in a high divalent ACSF (Supplementary Figs. S1 and S2).<sup>1</sup>

Unless stated otherwise, Parv<sup>+</sup>-mediated uIPSCs were measured with “high Cl<sup>-</sup>” KGluc pipette solution at a -65 mV holding potential to enhance responses. Som<sup>+</sup>-mediated uIPSCs were performed with normal K-Gluc pipette solution at a holding potential of -55 mV because the response polarity varied between recordings with the “high Cl<sup>-</sup>” version perhaps due to variability in diffusion of pipette solution into distal dendrites where Som<sup>+</sup> inhibitory synapses are located. DNQX (20  $\mu\text{M}$ ) and AP5 (100  $\mu\text{M}$ ) was added when measuring uIPSCs. A potential unitary connection was considered connected if the average response was >2 pA.

Coefficient of variation (CV) analysis was performed only on uEPSCs the average of which was >5 pA. For Som<sup>+</sup> EPSCs, uEPSC5 was measured because of its larger amplitude compared to uEPSC1. The CV was calculated as follows:  $(\text{StdDev}_{\text{Resp}} - \text{StdDev}_{\text{Noise}}) / (\text{avg response})$  (Beierlein et al. 2003; Faber and Korn 1991).  $\text{StdDev}_{\text{Noise}}$  was measured at baseline ~10–15 ms before uEPSC onset with the same fixed baseline-to-peak window employed for response measurements.

### mEPSC and Sr<sup>2+</sup> experiments

Miniature EPSCs (mEPSCs) were measured with TTX, picrotoxin, and AP5 in the bath. Strontium experiments were performed by replacing 2/2 mM Ca<sup>2+</sup>/Mg<sup>2+</sup> with 4/4 mM Sr<sup>2+</sup>/Mg<sup>2+</sup> (Gil et al. 1999). Asynchronous events induced by action potential trains in Sr<sup>2+</sup> were measured in a 400-ms window. For Parv<sup>+</sup>-mediated uIPSCs, the window started at 350 ms relative to train offset, and events were clearly not contaminated by spontaneously occurring events. For uEPSCs targeting Som<sup>+</sup> neurons, the window started at 40 ms, and we calculated that approximately five of six events were actual evoked quanta. Trains in Sr<sup>2+</sup> experiments were 50 Hz and typically between 15 and 20 APs in length for Parv<sup>+</sup> uIPSCs and Som<sup>+</sup> uEPSCs. Synaptic quantal events from both mEPSC and Sr<sup>2+</sup> experiments were analyzed using “Mini Analysis Program” (Synaptosoft) using a 9.5 and 7 pA detection threshold, respectively.

### Intrinsic membrane properties

Recordings examining the intrinsic membrane properties were performed with K-Gluc pipette solution.

### Som<sup>+</sup> bouton counts

In slice cultures made from GIN mice, single isolated excitatory neurons were transfected with DsRed using single-cell electroporation (SCE) (Haas et al. 2001; Rae and Levis 2002; Rathenberg et al. 2003). Approximately 8–10 days after plating, slices were removed from the incubator for 30 min for electroporation. SCE was accomplished using a glass micropipette filled with plasmid-containing solution. Resistance of the micropipette was between 15 and 20 M $\Omega$  when filled with saline containing (in mM) 149.2 NaCl, 4.7 KCl, 5 HEPES, and 2.5

CaCl<sub>2</sub>. A silver wire was placed inside the micropipette in contact with the 25 ng/ $\mu\text{l}$  DNA solution (pDS-Red2, Clontech). The insert containing the neocortical slice was placed in a customized chamber filled with tyrode solution containing (in mM) 150 NaCl, 3 KCl, 2 MgSO<sub>4</sub>, 10 HEPES, 10 dextrose, and 2 CaCl<sub>2</sub>. To prevent both the tip from clogging and the dilution of the DNA, positive pressure was applied to the pipette. The micropipette tip was slowly advanced toward the visualized cell while pipette resistance was constantly monitored with an applied square wave. When resistance increased ~25%, the SCE pulse protocol was performed. The electroporation pulse parameter protocol was a single train of 200 square pulses of 1-ms duration at 200 Hz with an amplitude of -5 V. Approximately two cells per slice expressed the plasmid. Robust expression occurred after 24 h, and both imaging and electrophysiological data indicated these cells were healthy after 10–14 days post transfection. Imaging confirmed that the neurons were excitatory by high spine density. After electroporation, slices were returned to the incubator. Two or 3 days later, TTX treatment was initiated.

After 5 days, GFP-positive axons/boutons and DsRed-positive somas/dendrites were imaged in live slices using two-photon microscopy ( $\times 40$ , Axioskop 2 FS, Zeiss). Specimens were excited with 910 nm light (Chameleon standard laser, Coherent), and optical sections were ~0.8  $\mu\text{m}$  thick, imaged with a 0.8  $\mu\text{m}$  spacing, and collected within 30  $\mu\text{m}$  of the slice surface. Two to three axons were chosen per image stack that were between 20 and 25  $\mu\text{m}$  in length. Boutons along an axon were identified by using a 1.5 $\times$  threshold brightness relative to the axon and by requiring that this increase be maintained for  $\geq 250$  nm (Fig. 8C). “Putative” synaptic contacts were identified based on a previous study (Di Cristo et al. 2004). A DsRed dendrite was analyzed across a 25  $\mu\text{m}$  length for any “putative” synapses. Each putative contact was determined by an overlap of  $\geq 500$  nm between the green and red signal in the plane for which the green signal was the highest (Fig. 8E). These criteria have been shown to accurately identify synapses ~82% of the time (Di Cristo et al. 2004). Laser power was set at a point where additional power did not reveal additional axons on spines. The experimenter was blind to the treatment history of the slices when images were collected and when analyzed.

### Drugs

Fast synaptic transmission was blocked with the following: N-methyl-D-aspartate (NMDA) receptor antagonist AP5 (100  $\mu\text{M}$ , Sigma), the AMPA/kainate receptor antagonists DNQX (20  $\mu\text{M}$ , Sigma) and NBQX (0.05  $\mu\text{M}$ , Sigma), and the GABA<sub>A</sub> receptor antagonist picrotoxin (100  $\mu\text{M}$ , Sigma). When examining uEPSCs, GABAergic transmission was enhanced with the GABA-receptor inverse agonist, zolpidem (0.1  $\mu\text{M}$ , Sigma). Chronic activity blockade was performed with tetrodotoxin (TTX, 2  $\mu\text{M}$ , Sigma).

### Analysis

All comparisons required data collection from “sister”-slice cultures originating from one preparation (1 animal). Statistical significance was  $P < 0.05$ , and all error bars in figures are SE. Unless otherwise stated, statistical comparisons were determined by the unpaired *t*-test (Mann-Whitney) and, for more than two groups, a one-way ANOVA followed by Fisher’s PLSD (multicomparisons). A  $\chi^2$  test was applied to determine changes in the percent of connected pairs, and a Fishers exact *P* value was used to determine significance. All statistics were performed with Statview software (SAS). Sample number (*n*) is either cell number or unitary connections tested and is always given in the following order: Control, TTX-treated. Numbers in graphs are sample number.

<sup>1</sup> The online version of this article contains supplemental data.

## RESULTS

To determine how activity regulates Parv<sup>+</sup> and Som<sup>+</sup> inhibitory circuitry, we examined neurons in slice cultures that had undergone 5 days of chronic action potential blockade by a pharmacological compound (TTX, 2  $\mu$ M). Electrophysiological properties were measured using simultaneous whole cell recordings of neighboring excitatory/inhibitory neuron pairs.

*Chronic activity blockade increases intrinsic membrane excitability of excitatory and inhibitory neurons*

Activity blockade caused an increase in input resistance in all three cell types examined (Parv<sup>+</sup>, Som<sup>+</sup>, Exc), with the greatest change occurring in excitatory and Som<sup>+</sup> neurons (75 and 63% increase,  $P < 0.0001$ , Supplementary Table S1), and comparatively little change in Parv<sup>+</sup> neurons (19%,  $P < 0.01$ ).

Previous studies have demonstrated that the intrinsic membrane excitability of both Som<sup>+</sup> and excitatory neurons increases with activity blockade (Desai et al. 1999b; Gibson et al. 2006). Increases in input resistance in all cells studied here are consistent with this process, and we have previously demonstrated that an increase in input resistance increases the excitability in Som<sup>+</sup> neurons in an almost identical experimental paradigm (Gibson et al. 2006). To see if the same is occurring for Parv<sup>+</sup> and excitatory neurons, we examined one metric for excitability—threshold current to evoke an action potential. Consistent with the increased excitability hypothesis, both Parv<sup>+</sup> and excitatory neurons had decreased thresholds (52 and 50% decrease,  $P < 0.0002$ ,  $279 \pm 33$  vs.  $134 \pm 18$  pA and  $82 \pm 6$  vs.  $41 \pm 3$  pA, Supplementary Table S1). Therefore all cell types examined in this study display increased intrinsic membrane excitability with chronic action potential blockade.

Excitatory neurons uniquely displayed a significant 26% decrease in capacitance ( $25.9 \pm 1.7$  vs.  $19.2 \pm 1.3$  pA,  $P < 0.004$ ,  $n = 71,58$ ), suggesting either that total membrane surface area is smaller after activity blockade or the intrinsic membrane capacitance is decreased. Very little change occurred in resting membrane potential for all cell types (see Supplementary Table S1 for all membrane properties).

*Local unitary excitatory synaptic input onto Som<sup>+</sup> and Parv<sup>+</sup> neurons is increased after chronic activity blockade*

We next examined local excitatory input onto Parv<sup>+</sup> and Som<sup>+</sup> neurons. Trains of action potentials were evoked in a presynaptic excitatory cell (5 APs, 20 Hz) and the resulting unitary EPSCs (uEPSCs) examined. We examined the first uEPSC in the train (uEPSC1), which represents “low-frequency” transmission at these synapses because the previous response occurred 8 s previously. As observed in acute slices, the uEPSCs targeting Parv<sup>+</sup> and Som<sup>+</sup> cells had different durations at half-height ( $4.5 \pm 0.9$  vs.  $13.8 \pm 3.7$  ms,  $P < 0.03$ ;  $n = 10,11$ ; uEPSC1) (Beierlein et al. 2003).

Both inhibitory neuron types showed a similar upregulation in excitatory input (Fig. 1, *A1* and *B1*). First, the percent of all cell pairs that had a detectable unitary connection, or “percent connected,” was increased for Parv<sup>+</sup> uEPSCs (Fig. 1*A2*). In addition, TTX induced a trend toward increased connectivity of Som<sup>+</sup> uEPSCs (Fig. 1*B2*), and a statistically significant increase was confirmed in additional experiments (see Fig. 5). Next, we measured the amplitude of the first uEPSC in the train (uEPSC1) to assay connection strength when a connection existed. Amplitude was dramatically larger in both cell types (Fig. 1, *A3* and *B3*). We then derived an excitatory drive value that was the average uEPSC1 amplitude including “nonconnections” (0 pA). By combining the effects of increased connectivity and increased amplitude, excitatory drive increased dramatically by approximately threefold for both inhibitory subtypes (Fig. 1, *A4* and *B4*). For Som<sup>+</sup> neurons, we were able to determine that these changes were likely due to an arrest of normal activity-dependent development. This is based on our findings that uEPSC properties measured in immature Som<sup>+</sup> neurons resemble TTX-treated mature neurons (Supplementary Fig. S3). Because increased excitatory drive induced by chronic activity blockade has previously been reported in excitatory neurons (Turrigiano et al. 1998), our data suggest that this may be a universal adaptation among all cortical neuron types.

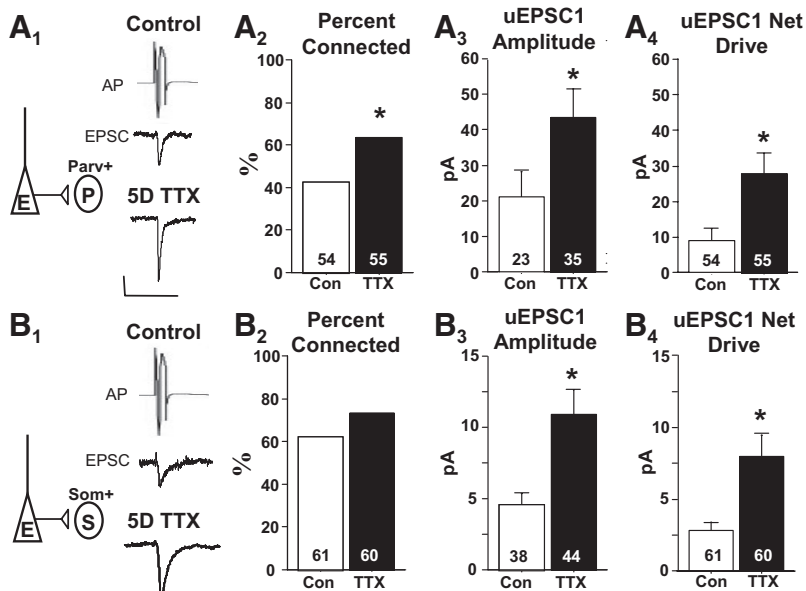


FIG. 1. Consistent with homeostatic regulation in inhibitory neurons, chronic activity blockade enhances excitatory drive onto both inhibitory neuron subtypes. *A1* and *B1*: unitary excitatory postsynaptic currents (uEPSCs) evoked from excitatory neurons (E) were examined in neighboring Parv<sup>+</sup> (P) and Som<sup>+</sup> (S) inhibitory neurons. *Top*: example of a presynaptic action potential (AP, truncated vertically). Note that APs are recorded in voltage clamp mode. *Bottom*: uEPSCs evoked in control and TTX-treated cultures. *A2* and *B2*: percent connected of all test pairs is increased for Parv<sup>+</sup> but not for Som<sup>+</sup> circuitry (but see Fig. 5*A* with larger data set). *A3* and *B3*: average uEPSC1 (1st uEPSC in a train) amplitude was dramatically increased in both subtypes. *A4* and *B4*: net excitatory drive, the average of both connected and nonconnected (0 pA) pairs, was increased at both subtypes. Scale bars: vertical, 700 and 10 pA for APs and PSCs, respectively. Horizontal, 50 ms. \* $P < 0.03$ . Sample number indicated in bars.

*Inhibitory synaptic transmission from Som+ and Parv+ neurons are differentially regulated by chronic activity blockade*

Next, we examined local inhibition in excitatory neurons provided by Parv+ and Som+ subtypes. Trains of action potentials were evoked in the presynaptic inhibitory neuron (8 APs, 20 Hz) and again, we first examined low-frequency transmission by focusing on uIPSC1 in the train. Parv+-mediated IPSCs were large and inward because we used a high Cl<sup>-</sup> K-Gluc pipette solution. But this was not possible for Som+ uIPSCs because with the same solution, the polarity of the uIPSCs was not consistent (see METHODS). Therefore we used a normal Cl<sup>-</sup> K-Gluc internal that resulted in outward Som+ uIPSCs.

Activity blockade differentially affected inhibitory connection strength based on inhibitory subtype (Fig. 2, A1 and B1). First, the “percent connected” of all cell pairs tested was decreased for Parv+ uIPSCs and unaffected for Som+ uIPSCs (Fig. 2, A2 and B2). No change in uIPSC1 amplitude was detected for either cell type (Fig. 2, A3 and B3). The net result of these changes is a 30% decrease in local inhibitory drive provided by Parv+ neurons and no change for that provided by Som+ neurons (Fig. 2, A4 and B4). The difference in pipette solution cannot account for the results because additional experiments using the identical pipette solution for both uIPSC types revealed the same differential regulation (Supplementary Fig. S2). While the rise time and width at half height of uIPSC1 were unchanged at Parv+ connections, the width of Som+ uIPSC1 was increased 22% ( $15.2 \pm 0.7$  vs.  $18.6 \pm 1.0$  ms,  $P < 0.02$ ,  $n = 25, 32$ ), suggesting that the total charge may have been enhanced in these responses. This latter alteration may be indicative of a change in subunit composition or our inability to effectively voltage clamp distally located Som+ synapses, but this was not examined further.

*Short-term plasticity of disinaptic, recurrent inhibition is differentially affected by chronic activity blockade conferring a frequency-dependent regulation*

Because the short-term dynamics of synaptic connections determines their information processing capabilities (Abbott

et al. 1997; Tsodyks and Markram 1997) and provides information about the pre- or postsynaptic locus of plasticity, we measured the short-term plasticity of both EPSCs and IPSCs with short stimulus trains (Figs. 3 and 4). Interestingly, while plasticity of synapses associated with Parv+ circuitry was unchanged with activity blockade (Figs. 3A and 4A), that of Som+ circuitry changed dramatically. Excitatory responses targeting Som+ neurons became much less facilitating (Fig. 3B, 1 and 2), and IPSCs originating from Som+ neurons became more depressing (Fig. 4B, 1 and 2). The last PSC in the train can be considered to represent high-frequency transmission because it followed the previous response by only 50 ms. Therefore the changes in short-term plasticity suggest that the relative magnitude of low- and high-frequency transmission was altered in Som+ circuitry. Specifically, there is a frequency-dependent shift in the relative contribution of Parv+- and Som+-mediated recurrent inhibition after chronic activity blockade where Som+ neurons contribute more at lower frequency network activity. The increase in short-term depression at Som+ uIPSCs appeared to be an induced change, and not a developmental arrest, as revealed by recordings before treatment (Supplementary Fig. S3).

*EPSC increases onto Som+ neurons are due to increased presynaptic release probability and synapse number*

We examined possible underlying synaptic mechanisms mediating the changes in excitatory synaptic function just described. First, consistent with the smaller set of data illustrated earlier (Fig. 1), additional recordings showed that the percent connectivity of uEPSCs onto Parv+ and Som+ subtypes was increased after activity blockade (Fig. 5, A1 and B1). If synaptic formation is a statistically independent process, this suggests that the increase in excitatory drive onto these cells is at least partially mediated by an increase in synapse number. However, investigation of action potential-independent miniature EPSCs (mEPSCs) revealed no change onto either Parv+ or Som+ neurons (Fig. 5A2 and B2). Either mEPSCs did not reflect changes in evoked transmission (Calakos et al. 2004; Reim et al. 2001; Sara et al. 2005) or any

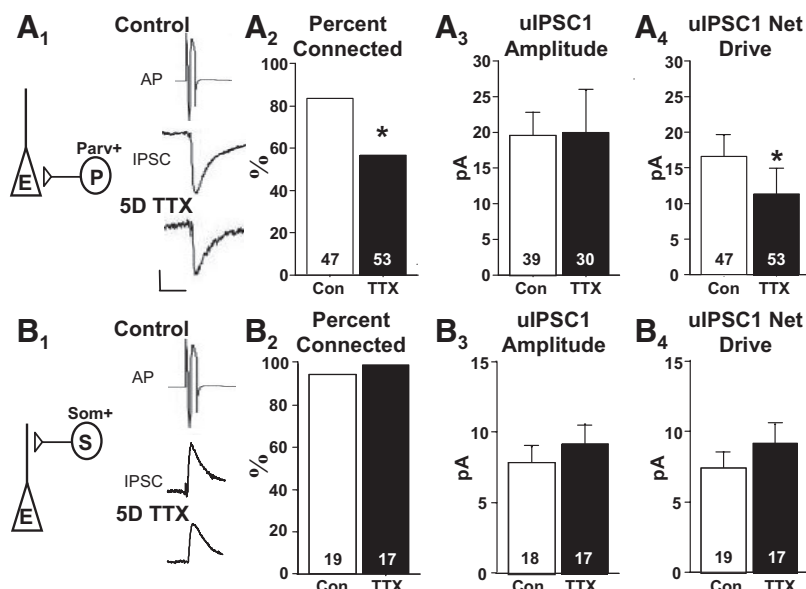


FIG. 2. Chronic activity blockade differentially regulates inhibitory synaptic drive onto excitatory neurons. A1 and B1: uIPSCs were evoked from Parv+ (P) and Som+ (S) neurons and examined in neighboring excitatory neurons (E). Parv+ uIPSCs were recorded using “high Cl<sup>-</sup>” pipette solution, and hence displayed downward waveforms. A2 and B2: percent connected of Parv+ and Som+ uIPSCs, but not Som+ uIPSCs, is decreased with chronic blockade (Chi-Sqr). A3 and B3: when a connection existed, average uIPSC1 amplitude was not affected for either inhibitory connection type. A4 and B4: net inhibitory drive was only decreased for Parv+-mediated uIPSCs. Scale bars: vertical, 700 and 10 pA for APs and PSCs, respectively. Horizontal, 50 ms. \* $P < 0.05$ .

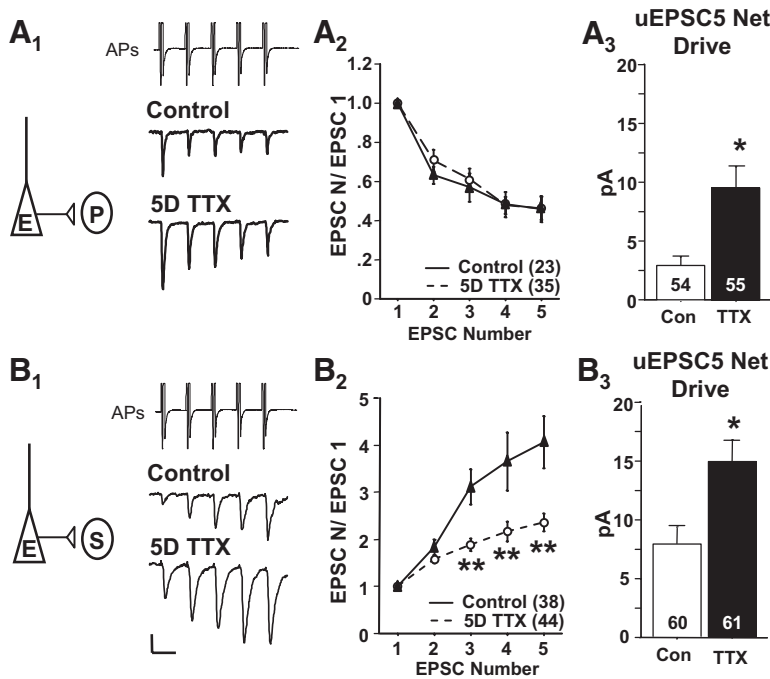


FIG. 3. Short-term plasticity is differentially regulated at excitatory synapses. *A1* and *B1*: recordings of uEPSC trains. *A2* and *B2*: Som+ uEPSCs became more depressing with TTX treatment while no change was detected in Parv+ uEPSCs. *A3* and *B3*: net excitatory drive at uEPSC5 was increased for both inhibitory neuron subtypes. Scale bars: vertical, 500 and 10 pA. Horizontal, 50 ms. \**P* < 0.02, \*\**P* < 0.004.

changes in the locally derived synapses mediating our measured uEPSCs are masked by compensating changes from other afferents.

We performed further analysis and experiments investigating the mechanisms of uEPSC changes, but we only focused on Som+ neurons because similar experiments in Parv+ neurons were more variable and inconclusive. First, to determine if increases in uEPSCs were consistent with increased quantal content (a property dependent on synapse number and release probability), we measured the coefficient of variation (CV) and failure rate of uEPSCs. In addition to increased synapse number after blockade, the decrease in uEPSC facilitation at Som+ neurons also suggested that release probability was increased.

Therefore quantal content should be higher, and we expected a lower CV and a decreased failure rate. This indeed was the case (Fig. 6, *A* and *B*). Finally, we measured the quantal amplitude of synapses comprising these uEPSCs by substituting  $Ca^{2+}$  with  $Sr^{2+}$ .  $Sr^{2+}$  induces asynchronous release of presynaptic vesicles enabling the measurement of individual quantal events and therefore the strength of individual synapses (Fig. 6*C*). Quantal amplitude was subtly decreased after blockade (Fig. 6*D*), and therefore cannot account for the increased uEPSC size. In conclusion, increases in excitatory drive onto Som+ neurons after chronic blockade are most likely due to an increase in quantal content that is mediated by increased presynaptic release probability and/or synapse number.

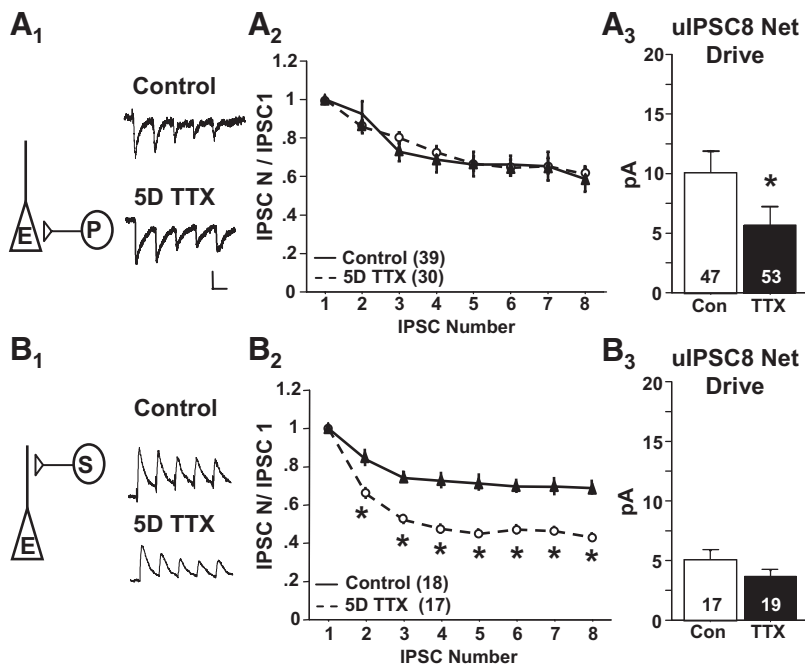


FIG. 4. Chronic activity blockade differentially regulates short-term plasticity of uIPSCs. *A1* and *B1*: uIPSC trains were evoked by trains of presynaptic action potentials (*top*). *A2* and *B2*: while short-term plasticity is unaltered with Parv+ uIPSCs, Som+ uIPSCs become dramatically more depressing. *A3* and *B3*: net drive for uIPSC8 is decreased at Parv+, but not Som+, synapses. Scale bars: vertical, 5 pA. Horizontal, 50 ms. \**P* < 0.004.

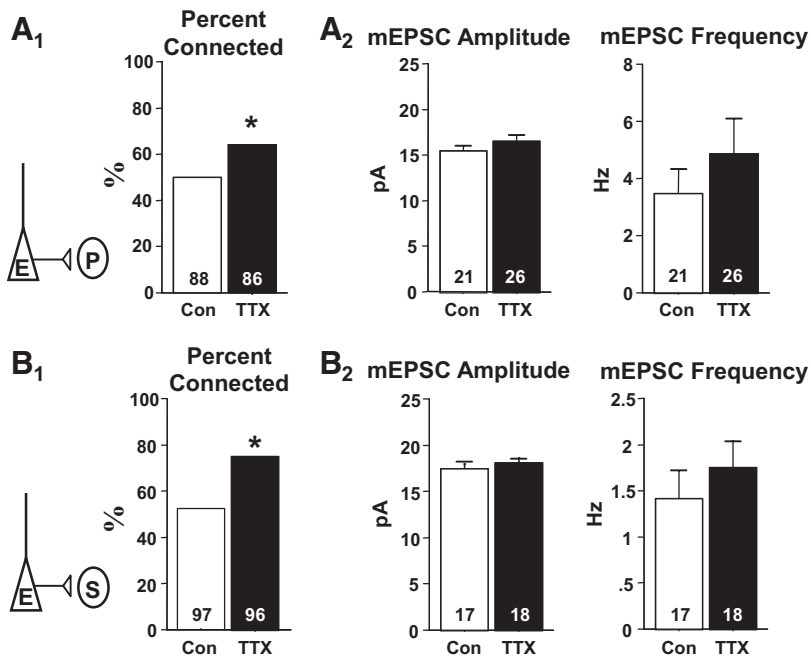


FIG. 5. Increased connectivity suggests an increase in synapse number mediating the increase in uEPSC drive. *A1* and *B1*: an increase in percent connected for all tested pairs suggests that increased synapse number is involved in the uEPSC increase. *A2* and *B2*: no changes were observed in mEPSC properties.

*Activity blockade induces opposing changes at Parv+ inhibitory synapses: increased release probability and decreased synapse number*

We next examined possible underlying synaptic changes at Parv+ mediated inhibitory synapses. Additional experi-

ments revealed a clear decrease in the percentage of connected pairs, which suggests a decrease in synapse number after chronic blockade (Fig. 7A). If the decrease is strictly mediated by synapse number, we would expect no change in the quantal amplitude. In support of this idea, measurements of quantal amplitude during  $Sr^{2+}$ -induced asynchronous release revealed no change (Fig. 7B). Therefore decreased Parv+ uIPSCs after chronic blockade are likely due to a decrease in synapse number, which is consistent with a previous anatomical study demonstrating decreases in Parv+ inhibitory synapse number after 5 day activity blockade in an almost identical preparation of neocortical slice culture (Chattopadhyaya et al. 2004).

Because short term plasticity of Parv+ uIPSCs was unaffected by chronic activity blockade, this appears to rule out any changes in presynaptic release probability (Fig. 4A). However, previous studies find that alterations in release probability are only detected at Parv+ uIPSCs using longer trains of action potentials (Kraushaar and Jonas 2000; Luthi et al. 2001). Therefore to better determine if Parv+ synapses undergo changes in release probability, we performed experiments using long trains (15 Hz, 750 pulses, Fig. 7C). With chronic activity blockade, Parv+ uIPSCs became more depressing during longer stimulus trains, implicating increases in release probability (Fig. 7C). Therefore increased release probability at Parv+ synapses may partially offset the effects of downregulated synapse number.

If increased release probability after activity blockade is masking the full functional effect of decreased synaptic number, then artificially increasing release probability closer to a ceiling should reveal greater decreases in Parv+ mediated uIPSCs. To test this idea, we increased release probability to ~80% of maximum by using 6 mM  $Ca^{2+}$  in the recording ACSF (Kraushaar and Jonas 2000). While Parv+ mediated uIPSCs observed in normal (2 mM  $Ca^{2+}$ ) displayed no difference in amplitude (Fig. 2A3), those recorded in 6 mM  $Ca^{2+}$  were decreased by about twofold after blockade (Fig. 7D).

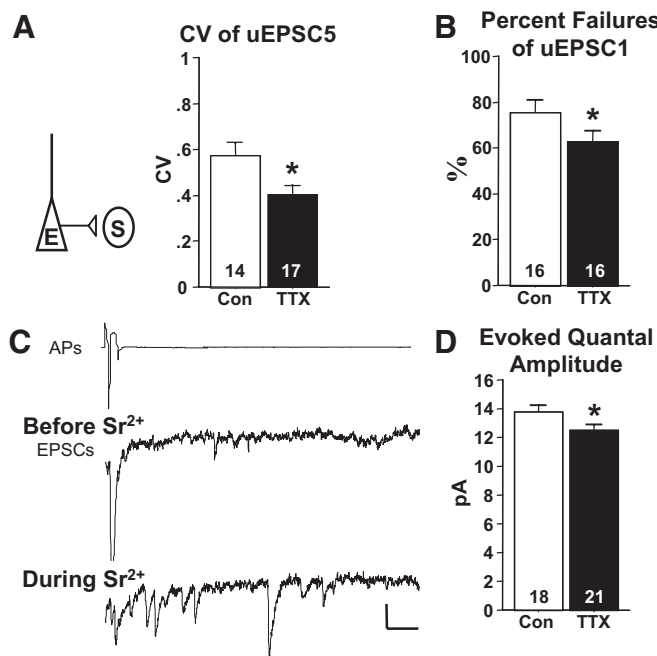


FIG. 6. Increased quantal content, not quantal size, mediating the increased strength of uEPSCs targeting Som+ neurons. CV (A) and failures (B) of uEPSCs are decreased with activity blockade suggesting increased quantal content. C: an experiment from control slices where  $Sr^{2+}$  was substituted for  $Ca^{2+}$  to induce asynchronous release. The AP shown is the last in a train of 15. The EPSC in the control trace is truncated. D: a small decrease in quantal size after chronic TTX treatment indicates that changes in synaptic efficacy, and probably postsynaptic receptor function, do not account for the increased excitatory drive. Scale bars: vertical, 1000 and 10 pA. Horizontal, 50 ms. \* $P < 0.05$ .



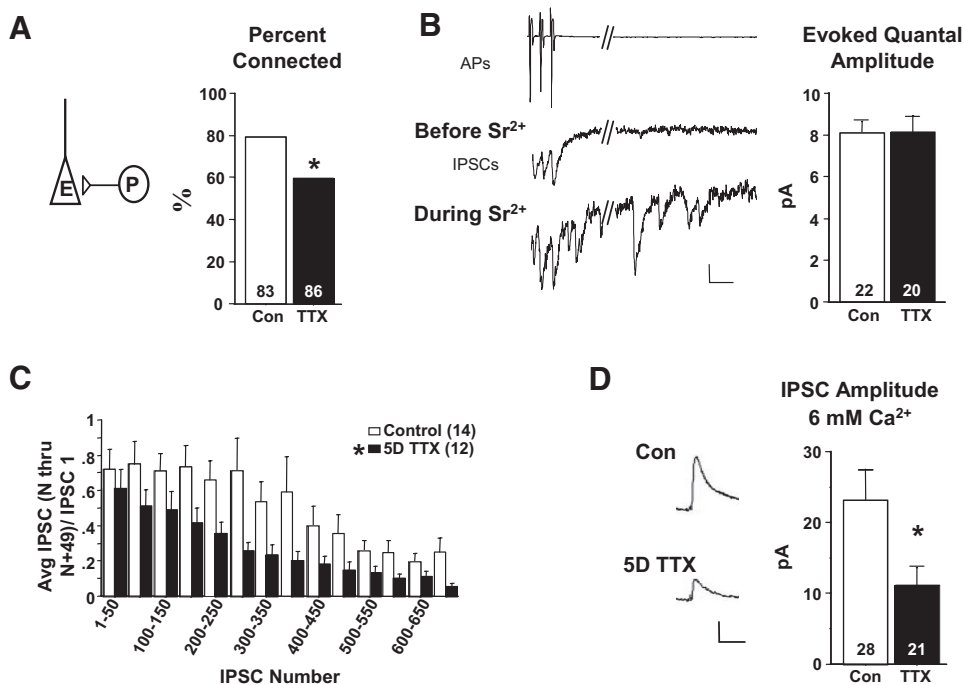


FIG. 7. With Parv+ uIPSCs, activity blockade does not affect quantal size but increases release probability. *A*: percent connected is decreased in all experiments performed examining Parv+ uIPSCs suggesting fewer synaptic contacts. *B*: Sr<sup>2+</sup> was substituted for Ca<sup>2+</sup> to induce asynchronous release and enable the measurement of quantal events directly mediating uIPSCs. The trace epoch shown during the last 3 action potentials in a train of 15. Quantal size was unchanged with TTX treatment, suggesting that regulation does not involve a postsynaptic efficacy mechanism (*n* = 22,20). Slashes represent 350 ms of omitted trace. Scale bars: vertical, 500 and 5 pA. Horizontal, 50 ms. *C*: long stimulus trains are more depressing after 5-day TTX treatment, suggesting an increase in release probability (*n* = 14,12). \**P* < 0.05, calculated with repeated-measures ANOVA. *D*: when release probability is increased to diminish the role of increased release probability, a decrease in uIPSC amplitude emerges (K-Gluc pipette solution used so currents are outward). Scale bars: vertical, 10 pA. Horizontal, 50 ms. \**P* < 0.03.

Therefore these data support the assertion that increased release probability and decreased synaptic number both occur with activity blockade, but functionally their effects offset each other. These data illustrate the diverse, and sometimes functionally opposing, changes that synapses undergo in response to chronic activity changes.

*Activity blockade also induces opposing changes at Som+ inhibitory synapses: increased release probability and decreased synapse number*

Functional measurements of Som+ inhibitory synapses also suggested the existence of opposing synaptic changes during activity blockade. The increase in short-term depression of Som+ uIPSCs suggested that release probability may be increased. This would be expected to increase uIPSC1, but

instead we observed no change (Fig. 2*B*, 3 and 4). Unlike Parv+ synapses, the percent of connected cell pairs was unchanged (Fig. 8*A*). However, there may still be decreases in the number of synapses per connection (see following text). To examine this possibility, we counted the number of Som+ (identified as GFP+) axon puncta, or swellings, and their contact frequency with excitatory neuron dendrites in live slices (Fig. 8; see METHODS). These were considered putative presynaptic boutons and putative synaptic contacts based on a previous study that validated this method in neocortical slice cultures from the same mouse line (Di Cristo et al. 2004) (Fig. 8*B*; see METHODS). Furthermore, other studies have used similar approaches of identifying putative synaptic contacts through visualization of closely opposed processes (Feldmeyer et al. 1999, 2002; Markram et al. 1997). To visualize and identify

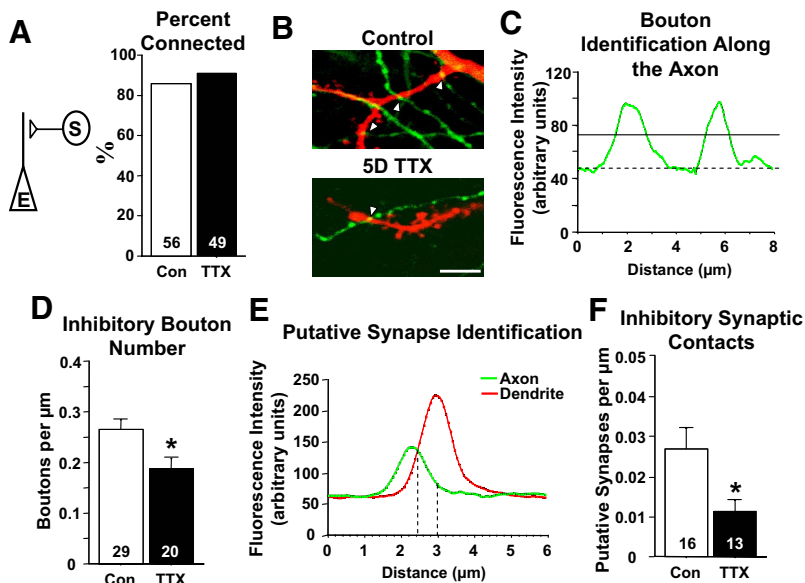


FIG. 8. A decrease in Som+ inhibitory “putative” boutons and synaptic contacts. *A*: no change in the percent connected for Som+-mediated uIPSCs after chronic activity for all pairs recorded. *B*: images of presynaptic axons and boutons (GFP) and postsynaptic excitatory neuron dendrites (DsRed). Putative contacts are indicated by arrow heads. Some axons travel in and out of the image plane. Each image is a superposition of images creating a 4.8- $\mu$ m-thick section, and hence some colocalized spots are not putative contacts because they are separated by >0.5  $\mu$ m in depth. Scale bars, 5  $\mu$ m. *C*: the GFP intensity distribution along an axon shows periodic increases that we define as “putative” presynaptic boutons. Dotted and solid horizontal lines represent baseline and threshold intensity levels. *D*: the density of “putative” presynaptic boutons is decreased with activity blockade (*n* = 29,20 images). *E*: intensity distributions of DsRed (red) and GFP (green) at a “putative” synaptic contact. Vertical dashed lines show the amount of overlap between the 2 distributions. *F*: “putative” synaptic contacts are decreased, also (*n* = 16,13 cells) \**P* < 0.02.

single isolated excitatory neurons, we expressed DsRed using single-cell electroporation. The number of “putative” synaptic contacts was quantified by counting the number of points that GFP+ puncta colocalized with DsRed+ dendrites (for criteria, see METHODS and Fig. 8E). A previous study observed that this method correctly identifies Som+ synaptic contacts 82% of the time (Di Cristo et al. 2004). Activity blockade reduced both the number of putative presynaptic boutons and synaptic contacts (Fig. 8, D and F). It is unlikely that these effects are due to decreases in GFP expression since soma GFP intensity was unaffected by TTX treatment ( $n = 56, 43$ ). Therefore chronic activity blockade reduces Som+ synapse number similar to Parv+ synapses.

However, unlike Parv+ inhibitory synapses the local connection frequency of Som+ synapses with neighboring neurons is maintained with chronic activity blockade. The reasons for this difference is unclear but may be due to a high number of Som+ synapses mediating a single unitary connection such that decreases in synapse number do not result in a detectable decrease in connection frequency. The high synapse number mediating connectivity is supported by the fact that Parv+ and Som+ uIPSC1 size is the same when the same pipette solution is used (Supplementary Fig. S2) even though Som+ uIPSCs would be expected to be smaller due to their preferred targeting of distal dendrites (Di Cristo et al. 2004; Somogyi et al. 1998).

In summary, these results suggest that Som+ uIPSC1 drive remains unchanged because of the offsetting affects of increased release probability and decreased synapse number. Because single quantal events evoked with  $\text{Sr}^{2+}$  substitution were unresolvable at this connection, changes in quantal amplitude or individual synaptic strength to the contribution of postsynaptic synapse strength to this regulation remains unknown. In summary, a reduction in synapse number and an increase in release probability are sufficient to explain the homeostatic alterations at both Parv+ and Som+ inhibitory synapses. However, a different functional outcome occurs in the two subtypes.

## DISCUSSION

Here, using paired recordings of synaptically coupled neurons in neocortical slice culture we have identified robust activity-dependent regulation of two inhibitory circuits. Chronic activity blockade induced increases in excitatory drive and intrinsic excitability in both Parv+ and Som+ inhibitory neurons demonstrating homeostatic plasticity occurs in all cell types, both excitatory and inhibitory. In contrast, there is differential regulation of uIPSCs from Parv+ and Som+ neurons in response to activity blockade. Net inhibitory drive originating from Parv+ neurons was decreased while that from Som+ neurons was unchanged. In addition, there was differential frequency-dependent regulation of Parv+ and Som+ circuitry. Short-term plasticity of both EPSCs and IPSCs associated with Som+ circuitry became less facilitating (or more depressing), whereas short-term plasticity (of short trains) of Parv+ synapses was unaffected. Therefore at low frequencies, Som+-mediated inhibition would be increased relative to Parv+. The synaptic mechanisms underlying these changes appeared to be primarily presynaptic release probability and synapse number as opposed to postsynaptic strength. Overall, our data illustrate that activity-dependent regulation of inhibi-

tion differs depending on cell type, which may be related to the different functions of these neurons.

A number of studies have examined activity-dependent, homeostatic regulation of synaptic and neuronal function. However, none have used paired recordings of synaptically coupled neurons involving identified inhibitory neuron types. Paired recordings allow a careful examination of evoked transmission of isolated, synaptically coupled neurons of known type. In addition, most studies have used dissociated neuron culture where, unlike the slice culture used here, circuit structure is not maintained.

### *Differential regulation of Parv+ and Som+ circuitry*

Homeostatic regulation of recurrent inhibition provided by Parv+ and Som+ neurons had two key differences. First, uIPSCs originating from Parv+ neurons were decreased while those from Som+ neurons were unchanged (Fig. 2). Moreover, the duration of uIPSCs from Som+ neurons was increased by 22%, suggesting that the net inhibitory charge provided by Som+ neurons may actually increase. Som+ inhibitory synapses preferentially target the dendrites of excitatory neurons (Di Cristo et al. 2004; Somogyi et al. 1998), indicating that aspects of dendritic inhibition may be paradoxically increased with activity blockade—an apparent nonhomeostatic adaptation. Because excitatory synaptic drive targeting excitatory neurons increases after chronic blockade (Turrigiano et al. 1998), increases in inhibition provided by Som+ neurons may increase in an effort to maintain a balance of inhibition and excitation in the dendrites.

Second, both uEPSCs and uIPSCs in Som+ circuitry underwent a shift in short-term plasticity while no such change occurred in Parv+ circuitry (in the context of short stimulus trains, Fig. 3 and 4). These adaptations indicate that a frequency-dependent shift occurs in the relative contribution of Parv+ and Som+ recurrent inhibition where Som+ inhibition is increased at lower frequency transmission. Recruitment of Som+ cell activity requires high-frequency firing of excitatory neurons and the subsequent facilitation of excitatory synapses (Gibson et al. 1999; Reyes et al. 1998). Therefore the frequency-dependent adaptation of Som+ synapses may be important to maintain any Som+ recurrent inhibition during low network activity as well as keep the proper balance of Parv+ and Som+ when activity is chronically decreased.

The synapses of Parv+ neurons are known to preferentially target the soma and proximal dendrites while synapses of Som+ neurons preferentially target distal dendrites (Di Cristo et al. 2004; Somogyi et al. 1998). Because of this differential targeting, the relative increase in Som+ inhibition at low frequencies would translate to a similar increase in distal or dendritic inhibition. In hippocampus, it has been demonstrated that low-frequency activity induces greater inhibition at the soma and high-frequency activity induces greater inhibition in dendrites (Pouille and Scanziani 2004), and it is likely that this distinction is due to Parv+ and Som+ neurons, respectively. Therefore our results suggest that the transition frequency from somatic to dendritic inhibition is reduced when activity levels are decreased, thereby changing information processing of disinaptic inhibitory circuitry.

*Mechanistic alterations at both Parv+ and Som+ inhibitory synapses are similar but result in different functional consequences*

Our data indicate that activity blockade induces both an increase in release probability and a decrease in synapse number at both Parv+ and Som+ inhibitory synapse subtypes. These are opposing effects that almost completely offset each other at Som+ inhibitory connections and only partially offset each other at Parv+ connections (Fig. 7). Hence different functional outcomes occur at these two synapse types although the same underlying synaptic changes appear to be occurring.

The decreases in synapse number were revealed at Parv+ inhibitory synapses by a decrease in connectivity with excitatory postsynaptic targets (Fig. 2A2). This is consistent with an earlier anatomical study performing the identical experiment (same age, except in visual cortex slice cultures) where Parv+ bouton number was decreased ~50% after 5-day TTX treatment (Chattopadhyaya et al. 2004). This value closely matches the drop in Parv+ uIPSCs when we removed the offsetting effect of release probability (52%, Fig. 7D), supporting the assertion of a synapse number decrease. The decrease in Som+ inhibitory synapse number was observed directly by counting their boutons. Decreased immunocytochemical inhibitory synapse markers after TTX treatment have been reported in dissociated cultures (Hartman et al. 2006; Kilman et al. 2002), but these studies did not distinguish between different inhibitory subtypes or make a link with evoked transmission.

Activity blockade also resulted in an apparent increase in presynaptic release probability at inhibitory synapses that was observed by changes in short-term synaptic plasticity of Som+ uIPSCs in response to short trains of stimulation (Fig. 4B) and of Parv+ uIPSCs in response to long trains of stimulation (>150 APs; Fig. 7C). Our assertion that release probability is increased at inhibitory synapses with activity blockade is mainly based on short-term plasticity measurements, and therefore we cannot completely rule out other pre- or postsynaptic mechanisms. The more pronounced decrease in Parv+ uIPSC amplitude measured in high  $Ca^{2+}$  after activity blockade is consistent with an increase in release probability (Fig. 7D).

Unchanged Parv+ IPSC quantal size (Fig. 7B) is in contrast with findings of increased inhibitory quantal size in dissociated cultures undergoing chronic TTX treatment (Hartman et al. 2006; Kilman et al. 2002). Several differences may account for this: 1) type (dissociated vs. slice culture) and age of culture (Burrone et al. 2002; Chattopadhyaya et al. 2004; De Simoni et al. 2003; Hartman et al. 2006; Wiring et al. 2006), 2) treatment length (5 vs. 2 days), and 3) more specific identification of inhibitory subtype in this study. Because quantal events could not be resolved at Som+ inhibitory synapses, we cannot rule out a postsynaptic efficacy change at this connection.

*Activity blockade causes similar increases in excitatory synaptic drive onto Parv+ and Som+ inhibitory neurons*

As previously observed at excitatory neurons, excitatory synaptic drive onto both Parv+ and Som+ inhibitory neurons increased in response to activity blockade (Fig. 1) (Lissin et al. 1998; Murthy et al. 2001; Turrigiano et al. 1998). Interestingly, it was reported that inhibitory neurons do not show excitatory

drive changes with activity blockade when only mEPSCs are examined (Turrigiano et al. 1998) (also shown in Fig. 5, A2 and B2). This highlights the importance of using paired recordings to examine evoked transmission because we were able to discern changes with this method.

We focused on mechanisms underlying the increase in excitatory drive onto Som+ neurons because this connection was most amenable to investigation. Similar to inhibitory synapses, activity blockade resulted in changes in presynaptic release probability and synapse number as opposed to quantal content. At Som+ neurons uEPSC increases are likely to be due to increases in both presynaptic release probability and synapse number as revealed by altered short-term plasticity (Figs. 3 and 6) and increased percent connectivity (Fig. 5B1), respectively. Decreases in failure rate and CV (Fig. 6) support an increase in release probability, synapse number, or both. In contrast, short-term plasticity of uEPSCs onto Parv+ neurons was unaffected by activity blockade suggesting no change in release probability occurred (Watanabe et al. 2005), but connectivity frequency did increase suggesting an increase in synapse number (Figs. 3A2 and 5A1). The increased release probability at excitatory synapses targeting Som+ neurons is consistent with more direct measurements of increased release at excitatory synapses targeting hippocampal excitatory neurons after chronic TTX treatment (Murthy et al. 2001).

*Effects of activity blockade are likely not due to cell or dendritic size changes*

It is unlikely that uEPSC alterations are a secondary effect due to morphological changes in inhibitory neurons. There are no alterations in the Som+ dendritic tree with TTX-treatment of slightly shorter duration (4 days) (Gibson et al. 2006). Similarly, Parv+ neurons do not display any gross alterations in soma size or in dendritic and axonal arbors after 5 days of TTX treatment in visual cortical slice culture at our experimental age (Chattopadhyaya et al. 2004). We found no change in membrane capacitance of Parv+ or Som+ neurons, suggesting that cell size was unchanged.

A possible morphological alteration in excitatory neurons is suggested by the 26% decrease in total membrane capacitance of excitatory neurons—an indication that these cells may be slightly smaller. But this change cannot fully explain why we observe differential regulation of amplitude or of short-term plasticity at Parv+ and Som+ inhibitory synapses.

*Universal aspects of cortical homeostasis and the control of network activity*

In response to activity blockade, excitatory neurons undergo an increase in their excitatory drive and in their intrinsic excitability (Desai et al. 1999b; Murthy et al. 2001; Turrigiano et al. 1998). Here we demonstrate that inhibitory neurons display the same adaptations, suggesting that these are universal adaptations for firing rate made among most cell types in cortex. Assuming that homeostatic regulation is intended to maintain firing at some set point, all cells appear to adapt to chronic decreases in activity by facilitating their firing to regain this set point.

If the same upregulation of activity occurs in both excitatory and inhibitory neurons, how is network activity increased after

blockade? And how are the principal players in information processing and relay (the excitatory neurons) regulated in this scenario? Network activity increases in spite of inhibitory circuitry activity being promoted (Corner and Remakes 1992; Gibson et al. 2006; Turrigiano et al. 1998). The critical point of regulation may be at the inhibitory synapse and, according to our study, the Parv+ inhibitory synapse. Because activity blockade induces a decrease in monosynaptic Parv+ inhibition (as described here) and an increase in monosynaptic excitation (Turrigiano et al. 1998), there is a net increase in the excitatory-to-inhibitory ratio impinging on excitatory neurons, which could underlie the increase in network activity. Parv+ synapses are optimally positioned to gate and control action potential generation at excitatory neurons since they target the soma and proximal dendrites (Somogyi et al. 1998). Therefore regulation of Parv+ inhibitory synapses provide an effective means to control the firing output of the principal neurons (the excitatory neurons) (Miles et al. 1996) and is an effective site for homeostatic regulation of activity.

## GRANTS

This research was supported by the National Institute of Neurological Disorders and Stroke Grant NS-045711 to K. M. Huber and supported by Fragile X Research Foundation funding to J. R. Gibson and K. M. Huber.

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